Doctor Kilgore's is that the sprinter's heart was undoubtedly of a sinus rhythm. Had he been working under an arrhythmia, I am sure he would not have gotten very far on the cinder pathway without going into a heap. The faster a fibrillating heart goes the greater is its arrhythmia and the more damage to its reserves. Surely, Doctor Kilgore would not try to tell us that the slowing of a pulse rate and the lengthening of its diastolic period is not good treatment, and an important point to observe in recovery from decompensation.

THE ASCHHEIM-ZONDEK TEST FOR PREGNANCY*

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DISCUSSION *by Gertrude Moore, M. D., Oakland; Alvin G. Foord, M. D., Pasadena; George D. Maner, M. D., Los Angeles.*

THE early work of Aschheim and Zondek[1] on sex glands and sex hormones progressed slowly because the rabbit was the only known suitable animal for the purpose. Rabbits became scarce and expensive, and some other animal had to be found. They soon found that immature white mice and immature white rats were suitable.

Working with immature female white mice they[2] showed that ovarian hormone in the blood or in the urine of a woman does not indicate pregnancy, and that fairly large quantities of ovarian hormone can be demonstrated in the blood and in the urine of women in many conditions besides pregnancy. They found that presence in the blood and in the urine of a substance which in the test animal causes hemorrhagic follicles and cellular proliferation in the ovaries, and probably premature ovulation like the reaction following the injection of an extract of the anterior portion of the pituitary gland, indicates pregnancy. They considered this substance to be a hormone secreted by the anterior portion of the pituitary gland.

VALUE OF STUDIES OF ASCHHEIM AND ZONDEK

Aschheim and Zondek[3] then showed that the test is practically specific for pregnancy by applying it to many other conditions with negative results. Twenty-six urines of normal, menstruating women all gave negative results. Six urines from old women gave negative results. Sixteen urines of unknown source gave results that agreed with the clinical findings. Three urines from women with irregular menstruation gave negative results. Of sixteen urines from men, one gave a positive result which could not be explained. Fifteen urines from women with internal diseases gave negative results. One urine from a woman with cystitis gave a positive result. Of three urines from acromegaly, two were highly toxic; they killed all the animals and one gave a positive result. Thyroid diseases gave negative results. Of twelve urines from women with infections of the genital organs, ten gave negative results and two gave positive results, and one of these had received Roentgen-ray treatment. Ten benign ovarian tumors and eighteen uterine myomas gave negative results. One case of uterine myoma gave a positive result and was found to be pregnant. Of sixty urines from women with cancer, fifty-eight gave negative results and two gave positive results. The two urines that gave positive results came from women with carcinoma of the ovary. Of 197 urines from normal pregnant women, 195 gave positive results and two gave negative results, and these gave positive results a few days later. After delivery, the results were always negative in eight days and frequently in five. After death of the fetus in utero, and after abortion the results became negative as soon as after normal delivery. In tubal pregnancy the results were positive while the fetus was alive, but soon became negative after the death of the fetus. Two urines from women with hydatidiform mole gave positive results.

In 1929, Friedman[4] reported on thirty-six urines from pregnant women, all of which gave positive results in rabbits.

OUTLINE OF AUTHOR'S STUDIES

The work reported here was done on rabbits, and the following questions are considered:

1. The most suitable age of the rabbit.
2. The time required for the reaction in the rabbit.
3. The Aschheim-Zondek test with urine from normal men and with urine from normal nonpregnant women.
4. The Aschheim-Zondek test in pregnancy.
5. Preservation of urine for the Aschheim-Zondek test.
6. The Aschheim-Zondek test in various pathologic conditions.

The Age of the Rabbit.—Female rabbits two months old, from three to four months old, and some adults were injected with urine from the same pregnant woman. The rabbits between three and four months old reacted better than the younger or older rabbits. I prefer rabbits between three and four months old.

The Reaction in the Rabbit.—A number of rabbits were inoculated at about the same time with urine from a pregnant woman; then some were examined after twenty-four hours, some after forty-eight hours, some after seventy-two hours, some after five days, some after seven days, some after ten days and some after twenty-one days. Twenty-four hours after injection there was a slight reaction in the ovaries; it was good after forty-eight hours; it reached its height in four or five days, then gradually subsided, leaving scarred masses with a few hemorrhagic points by the end of twenty-one days.

Urines from Normal Persons.—Five urines from normal men, five from normal nonmenstruating women, and five urines from five menstruating women all gave negative results.

Urines from Pregnant Women.—All rabbits were between three and four months old, and for diagnostic test each rabbit received about 12 cubic centimeters of urine, with a specific gravity of

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1,020 intravenously, and was killed for examination two or three days after the injection. More than one hundred urines from pregnant women have been tested to date, and all gave positive results. On titrating some of these urines for hormone content, the highest titers were obtained fairly early in the pregnancy. Urines from women two months pregnant usually contained about five rabbit units per cubic centimeter (0.2 cubic centimeter of urine usually produced a fair reaction). In the fourth or fifth months, the urine contained two or three units per cubic centimeter, while at full term the urine contained about one unit per cubic centimeter. Of five patients who were examined after delivery all gave negative results with 12 cubic centimeters of urine, the usual test dose in five days. Urine usually contained twice as much hormone per cubic centimeter as did blood serum.

Tubal Pregnancy.—Four urines from women with ruptured tubal pregnancy were examined; two gave positive results and two gave negative results.

Abortion.—Four urines from women with incomplete abortion were examined; two gave positive results and two gave negative results.

Preservation of Urine. — Urine preservative tablets as supplied by life insurance companies were added to three urines from pregnant women, and the urines were kept in a dark place at room temperature. A year after having been voided these urines still gave positive results, but the titer of each urine had dropped from more than five rabbit units to about one rabbit unit per cubic centimeter.

THE TEST IN RELATION TO VARIOUS PATHOLOGIC CONDITIONS

Irregular Menstruation.—One urine from a woman with irregular and abnormal menstruation gave a negative result.

Chorio-epithelioma.—One case of chorio-epithelioma came under observation. The women expelled a hydatidiform mole and the urine of the patient gave a strongly positive result with the Aschheim-Zondek test. After expulsion of the mole the uterus remained large, there was much bleeding, and in three weeks the Aschheim-Zondek test had not become negative. The uterus was removed and showed extensive growth of chorioadenoma. The Aschheim-Zondek test became negative in a few days and was still negative five months after the hysterectomy. At the present time, three years after the hysterectomy, this patient appears to be perfectly well.

Systemic Diseases.—Three urines from women with diabetes and two urines from women with syphilis gave negative results.

Acute and Chronic Inflammations.—Two urines from women with mastitis, five urines from women with endometritis, five urines from women with appendicitis, and five urines from women with acute salpingitis gave negative results.

Thyroid.—Five urines from women with thyroid disorders gave negative results.

Benign Growths.—Ten urines from women with fibromyomata of the uterus were tested. Seven gave negative results and three were positive. In the uteri of those giving positive results, remnants of placental tissue were found which indicated recent abortion. These cases were operated on because of sudden severe hemorrhage. Five urines from women with ovarian cysts, one urine from a woman with teratoma of the ovary, and one urine from a man with teratoma of the testicle gave negative results.

Malignant Growths.—Three urines from women with carcinoma of the ovary, five urines from women with carcinoma of the uterus, five urines from women with carcinoma of the breast and three urines from women with carcinoma of the stomach gave negative results.

SUMMARY

Rabbits from three to four months old reacted better to the pregnancy hormone than did younger rabbits or older rabbits.

The reaction in the rabbit's ovaries developed slowly and subsided more slowly. It required from four to six days to reach its height and about a month to subside.

False positive results were not obtained with urines from normal men and with urines from normal nonpregnant women.

Urines from more than one hundred pregnant women all gave positive results. I have not yet obtained a false negative result.

Urines from pregnant women received preservative and were kept for one year. One year after having been voided they still gave good positive results, but had lost 80 per cent of their original titer.

Positive results were not obtained with urines from men and women suffering from various pathologic conditions, except in hydatidiform mole and in chorio-epithelioma.

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DISCUSSION

GERTRUDE MOORE, M.D. (2404 Broadway, Oakland).—That the clinician has learned to rely on the present-day hormone test for pregnancy, is increasingly evident. The test, when properly performed, justifies this confidence; for it is possessed of a high degree of accuracy, equal to that of most laboratory procedures. Depending as it does on the presence of living chorionic cells in contact with the circulation, it is not surprising that the highest titers are reached, not in normal pregnancy, but in those malignancies in which the neoplastic tissue is a new growth of chorion derivatives. It is, therefore, very positive in hydatidiform mole, and in chorio-epithelioma of either the male or female sex glands. Positive findings occur in

pregnancy only while there is living chorion in contact with the maternal circulation, becoming negative rather promptly upon the separation or disintegration of this structure. For this reason the test is positive in almost 100 per cent of normal intra-uterine pregnancies, but falls far short of this ideal in ectopic pregnancy, as is noted in Doctor Ruediger's series. In tubal pregnancy, tubal abortion or rupture with death of the fetus and disintegration of the product of conception is not uncommon. Similarly, in intra-uterine pregnancy, a complete separation of the product of conception from the maternal wall may occur, with a negative reaction resulting, even though the sac has not been expelled. A dead fetus with living chorion attached to the uterine wall continues to give positive reaction. I would like to stress a few practical points, the performance or lack of performance of which makes the difference between a reliable result and one of questionable value.

The selection and care of the rabbits used in the test have an importance not to be overlooked, if we would have the highest attainable accuracy. The habit of some workers of buying rabbits of unknown age and mating history cannot be too strongly condemned. Injecting one such animal, and relying entirely upon the results obtained, is not the highest type laboratory procedure. The fact that fair results are obtained does not justify us in an indifference to the possibilities of materially improving the test if all possible safeguards are thrown around the procedure. The animal's age must be known in order to be sure of sexual maturity, for immature ovaries do not react. They must be kept in isolation, preferably for thirty days, since contact with animals, even of the same sex, may induce ovulation and destroy the value of the test. At least two animals should be used to each test, for it has been clearly demonstrated that about three and one-half per cent of mature rabbits are refractive to the hormone, and if used alone will give false negative reactions.

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Alvin G. Foord, M. D. (Pasadena Hospital, Pasadena). Doctor Ruediger's results coincide, I am sure, with those of others doing sufficient numbers of tests to warrant conclusions. His selection of rabbits between three and four months of age is a point of great importance. Particularly is the use of too young rabbits to be condemned. Some of the earlier failures in using the rabbit test were due to this mistake. Another occasion for false negatives in the presence of pregnancy is the tendency of some physicians, usually at the insistence of their patients, to ask for a test too early in the pregnancy, such as one to three days after the menstrual period has been missed. Since it requires a certain length of time for sufficient hormone to be developed and excreted, it is best to wait at least ten days after the missed menstrual period before doing the test. In a small number of cases where the exact length of time between coitus and the rabbit test is known, it has been found that positive reactions cannot be expected before twenty-one days. In the case of a wife of a physician, however, when the test was made in our laboratory, where the time was accurately estimated, a positive Friedman result was obtained sixteen days after coitus, and subsequent obstetric history verified the time factors.

I personally believe, as does Doctor Ruediger, that false positives in non-pregnant women are usually due to laboratory error. Some of the earlier figures reported include such mistakes. In cases of teratoma testis, positive reactions with the rabbit are rare. The animal is too big and not sensitive enough for the amount of hormone passed in the urine. The mouse should be used instead, using Ferguson's technique or some other concentration method.

In conclusion, I wish to state that, inasmuch as a different animal is used, it appears to me only just that the rabbit test be given the name Friedman test, and that the mouse technique be called by its original name—the Aschheim-Zondek test.

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George D. Maner, M. D. (657 South Westlake Avenue, Los Angeles). — The author entitles his paper "The Aschheim-Zondek Test for Pregnancy," but in the text refers to the use of rabbits; so I presume the test under discussion is, in reality, the Friedman modification of the Aschheim-Zondek test. The fundamental principles underlying both are the same, viz., production of changes in the gonads of the test animals by a gonadotropic hormone present in pregnancy urine. In the Friedman modification, the urine is injected intravenously into female rabbits.

The amount of urine within certain limits used seems to be immaterial—amounts varying from 5 to 20 cubic centimeters having been used by different observers; but it seems that the greater number of workers prefer from 10 to 22 cubic centimeters doses. Single injections of this amount, or multiple injections of split doses have been used with remarkably uniform parallel results. Either preadolescent, adolescent, or adult doses, may be used. In adult animals, experimental data show that ovulation is complete in ten hours; and some few workers even record their final reading or report at the end of eighteen to twenty hours, while others prefer longer periods such as thirty-six to forty-eight hours. Thus it appears that the time elapsed after injection does not have much bearing upon the accuracy of the test. I am in accord with the author that it is best to wait forty-eight hours before reporting final results.

The author brings out an important practical point in regard to the preservation of the urine specimens, and the maintenance of the hormone content in high concentrations. Another important and interesting observation is in his findings of high concentrations of hormone (amounts equivalent to 0.2 cubic centimeter) in early pregnancy. This is important and should be borne in mind, in that one finds frequent reported statements in the literature that "positive reactions in amounts less than one cubic centimeter urine" or "in amounts one-twelfth the usual dose of 10 cubic centimeters," are significant and constitute strongly presumptive evidence of molar pregnancy or chorio-epithelioma.

The author is quite fortunate in not having any false or incorrect negative reactions in his series, inasmuch as a small percentage of rabbits, for some unknown reason, are refractory to the hormone.

I regret that the author did not go more into detail as regards the application or limitations of the test to the diagnosis of ectopic pregnancy. I wonder if the two negative tests in his series were considered as incorrect negatives, or whether tubal rupture or abortion had occurred before the urine was obtained. In the latter instance one would expect the test to be negative in that the chorionic epithelium was either nonviable or not in contact with the circulation of the host. By the Friedman test a negative reaction may be obtained in about twenty-four hours after the separation of the placenta, in both uterine and extra-uterine pregnancy.

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Doctor Ruediger (Closing). — Some credit is usually given the originator of a test. Aschheim and Zondek showed that during pregnancy a hormone is excreted in the urine which, when injected into other suitable female animals, produce a marked reaction in the ovaries. According to Aschheim and Zondek, the female rabbit was the first animal used; but rabbits were expensive. They then found that immature white mice and immature white rats also were suitable for the test, and they used mice because these were the cheapest.

Widal used the agglutination test as a diagnostic test for typhoid fever, and although the present agglutination test differs greatly from Widal's original test, it is frequently called the Widal test.

Wassermann used the complement fixation test as a diagnostic test for syphilis. This test was called the Wassermann test. Modifications have been many, but the test in general is still known as the Wassermann test. In my opinion, somewhat modifying or developing a test cannot be considered originating a new test.

Contrary to Doctor Maner's experience or opinion, I have not obtained negative results twenty-four hours after the birth of the child; negative results were obtained five days after delivery.

In the cases of tubal pregnancy all had ruptured, some probably fourteen days before the operation.